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# POSTER ABSTRACTS

## 113.SICKLE CELL DISEASE, SICKLE CELL TRAIT AND OTHER HEMOGLOBINOPATHIES, EXCLUDING THALASSEMIAS: BASIC AND TRANSLATIONAL

### Elevated Heme Drives Evoked and Ongoing Pain Behaviors in WT Mice By Sensitization of Dorsal Root Ganglia Neurons

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Individuals with sickle cell disease (SCD) suffer from complex stimulus-evoked and ongoing pain that is mediated in part by the hyperexcitability of their peripheral sensory neurons. However, it is unclear whether acute or chronic elevation of cell free heme, a key pathological feature of SCD, contributes to the aberrant activity of sensory neurons. Thus, determining whether heme drives pain behavior and enhanced excitability of dorsal root ganglia (DRG) primary sensory neurons involved in pain signal transduction is critical for the development of targeted pain therapies for SCD. In the current experiments, we use evoked and ongoing pain behavior assays and *in vitro* calcium imaging to evaluate the hypothesis that acute increases in heme causes direct sensitization of nociceptive DRG neurons in wildtype (WT) mice. Our preliminary data reveal that similar to pain behaviors in the mouse model of SCD, hind paw injection of heme into WT mice induces mechanical hypersensitivity **(Figure 1)**. We also observed increased aversive responses to dynamic paintbrush and noxious needle stimulation as well as the onset of paw licking behavior suggesting that heme also drives ongoing, non-evoked pain. These data suggest that axon terminals in the skin become rapidly sensitized when exposed to elevated heme. We next administered heme to primary cultured DRG neurons *in vitro* and observed calcium flux across the neuronal cell membrane that was dependent on extracellular calcium. To determine if heme directly triggers action potentials or enhances spontaneous activity of sensory neurons, we conducted electrophysiological recordings of neuronal activity in neurons held at resting membrane potential at physiological temperature. Our preliminary data indicate that exposure to heme may directly enhance neuronal action potential firing frequency **(Figure 2)**. Together, these data suggest that heme opens calcium channels on sensory neurons to initiate pain signal transduction and drive acute pain behaviors. We plan to conduct additional non-evoked pain behavior assays including facial grimace analysis and conditioned place aversion to determine whether heme injection is sufficient to drive ongoing pain. We will further determine whether exposure to heme enhances the intrinsic excitability of mechanical and cold sensitive neurons as heme-dependent hyperactivity of these neurons may lead to our observed acute onset mechanical and cold hypersensitivity in heme-injected mice. Completion of these experiments will shed light on the contributions of cell free heme to SCD pain and illuminate downstream effectors that may be targeted to provide analgesic relief for this historically understudied and debilitating disease.

**Disclosures** No relevant conflicts of interest to declare.

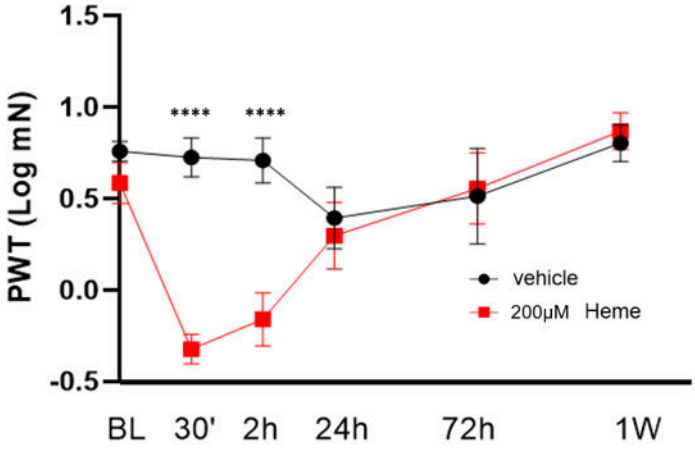


**Figure 1: Hind paw injection of heme induces acute mechanical hypersensitivity.** Following baseline mechanical sensitivity testing using the von Frey Up/down method, WT mice received a single hind paw injection of 200μM heme or vehicle. Paw withdrawal thresholds were significantly decreased at 30 minutes and 2 hours post injection, but not at later timepoints. Statistical significance was determined using a two-way ANOVA followed by Bonferroni's post-hoc test (n = 11-15 mice per group, **** P<0.0001)

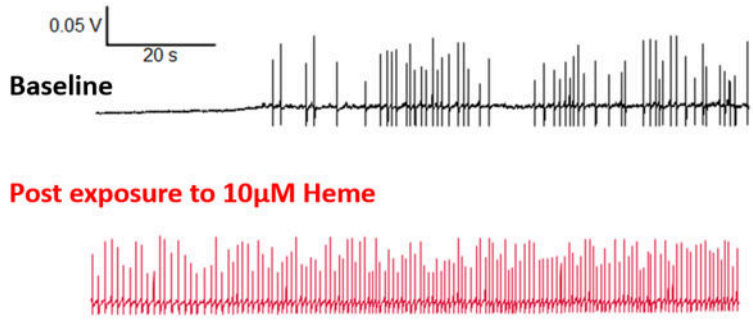


**Figure 2: Acute heme exposure enhances neuronal activity** Example trace of a primary cultured small-diameter DRG neuron before and after exposure to 10μM heme for 3 minutes.

**Figure 1**

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